

APPENDIX B

ALL CLAIMS AFTER ENTRY OF THE AMENDMENT HEREIN

1. A nucleic acid sequence for enhancing expression of a useful gene incorporated into a gene expression vector for enhancing expression of a useful gene comprising a nucleic acid sequence corresponding to a 5'-untranslated region of a viral gene or a fragment or a variant thereof, and is incorporated downstream of the expression regulatory promoter sequence and upstream of the first useful gene in a gene expression vector.

2. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

3. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of BoxA, BoxB, a trans factor-binding site, and a combination thereof.

4. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region further comprises an AUG or ATG sequence.

5. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises a part of or an entire region of IRES (internal ribosomal entry site) of viral mRNA.

6. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1 further comprises a portion of a coding region adjacent to the 5'-untranslated region, or a fragment or a variant thereof, of a viral gene in addition to said nucleic acid sequence.

7. Canceled

8. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid is a cDNA sequence.

9. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said gene expression vector is a vector for expression in eukaryotic cells.

10. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said virus is RNA virus.

11. The nucleic acid sequence for enhancing expression of a useful gene according to claim 10, wherein said virus is picornavirus.

12. The nucleic acid sequence for enhancing expression of a useful gene according to claims 10, wherein said virus is HCV (hepatitis C) virus.

13. The nucleic acid sequence for enhancing expression of a useful gene according to claim 10, wherein said virus is HCV virus, and said nucleic acid sequence for enhancing expression of a useful gene further comprises a portion of the coding region for the core protein of the HCV virus or, a variant thereof.

14. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of nucleotides 1-180 of SEQ ID NO: 1.

15. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of nucleotides 181-341 of SEQ ID NO: 1.

16. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of nucleotides 1-341 of SEQ ID NO:1.

17. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of nucleotides 181-713 of SEQ ID NO: 1.

18. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of nucleotides 1-713 of SEQ ID NO:1.

19. Canceled

20. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence corresponding to a region other than the 5'-untranslated region.

21. A nucleic acid sequence for enhancing expression of a useful gene incorporated into a gene expression vector for enhancing expression of a useful gene comprising a nucleic acid sequence of nucleotides 181-341 of SEQ ID NO: 1 having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof.

22. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1 or claim 21, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of its own translation promoting activity.

23. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1 or claim 21, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of accelerating IRES activity.

24. A nucleic acid sequence for enhancing expression of a useful gene comprising a nucleotide sequence of SEQ ID NO: 7, which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

25. A nucleic acid sequence for enhancing expression of a useful gene which comprises a polynucleotide having a similar IRES activity to an IRES activity of a nucleotide sequence of SEQ ID NO: 7, and consisting of a fragment or a variant of the sequence, which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

26. An isolated polynucleotide consisting of a nucleotide sequence of SEQ ID NO:7.

27. An isolated polynucleotide having a similar IRES activity to an IRES activity of a nucleotide sequence of SEQ ID NO: 7 and consisting of a fragment or a variant of said sequence.

28. A gene expression vector comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 1 or claim 21.

29. A host cell transformed or transfected with the vector according to claim 28.

30. A method of expressing a useful gene product using the vector according to claim 28.

31. A method for producing a useful gene product comprising the steps of: growing the host cell according to claim 29 in a medium; and isolating the useful gene product from the cell and/or the growth medium.

32. A method for enhancing expression of a useful gene product using the vector according to claim 28.

33. A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26.

34. A probe for screening IRES-dependent translation initiators, comprising the

polynucleotide according to claim 26.

35. A therapeutic composition for treating diseases resulting from reduction of cap-dependent mRNA translation in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 1 or claim 21 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

36. A therapeutic composition for treating diseases resulting from reduction of IRES activity in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 24 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

37. A method for determining the severity of hepatitis C, comprising the steps of: detecting the presence of a target polynucleotide sequence contained in a biological sample derived from a test subject, by using the polynucleotide according to claim 26 or claim 27 as the target; and determining the severity of the hepatitis C based on the presence of the sequence.

38. The nucleic acid sequence for enhancing expression of a useful gene according to claim 21 further comprising a nucleic acid sequence of nucleotides 1-180 of SEQ ID NO: 1 having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof.

39. The nucleic acid sequence for enhancing expression of a useful gene according to claim 21 further comprising a nucleic acid of nucleotides 342-713 of SEQ ID NO: 1 having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof.

40. The nucleic acid sequence for enhancing expression of a useful gene according to claim 21 further comprising a nucleic acid sequence of nucleotides 1-180 and 342-713 of SEQ

ID NO: 1 having one thymidine inserted into position 207 of SEQ ID NO: 1, and a fragment or variant thereof.

41. A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 27.

42. A probe for screening IRES-dependent translation initiators, comprising the polynucleotide according to claim 27.

43. A method for determining the severity of hepatitis C, comprising the steps of: detecting the presence of a target polynucleotide sequence contained in a biological sample derived from a test subject, by using the polynucleotide according to claim 27 as the target; and determining the severity of the hepatitis C based on the presence of the sequence.

44. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises a sequence corresponding to at least one region selected from the group consisting of a pyrimidine-rich tract, BoxA, BoxB, a trans factor-binding site, and a combination thereof.

45. The nucleic acid sequence for enhancing expression of a useful gene according to claim 44, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or a proximate sequence in at least one position corresponding to a pyrimidine-rich tract, BoxA, BoxB and/or trans factor-binding site contained in the 5'-untranslated region.

46. A therapeutic composition for treating diseases resulting from reduction of IRES activity in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 25 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.